Use of the spore photoproduct lyase (*splB*) gene as a marker for the detection and enumeration of spore-forming microorganisms

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Spore-forming microorganisms pose one of the largest problems in maintaining ultra-clean environments, such as spacecraft and their assembly facilities. Unique to spore-forming bacteria is the *splB* gene, which encodes the Spore Photoproduct Lyase enzyme. It is possible to evaluate the burden of spore-forming organisms in a given sample by quantitatively detecting the presence of *splB*. Thirty-five *Bacillus* strains were procured from various sources, and their DNA was extracted by both manual and automated methods. The 16S (*rrn)* and *splB* genes were PCR amplified, and species showing positive *splB* gene amplification were sequenced. Alignment of the *splB* sequences enabled the identification of highly conserved domains for the design of semi-degenerate "universal" *Bacillus splB* primers for PCR amplification of unknown samples.

The *splB* gene nucleotide sequence is highly heterogeneous and ~70% nucleotide sequence similarity was observed among various species of *Bacillus*, as well as between inter-genus spore-forming bacteria. Such heterogeneity of gene sequence has been exploited to design effective probe-primer sets specific for a given problematic species. For example, a specific TaqMan *splB* probe-primer set was synthesized that allowed us to perform quantitative real-time PCR to detect *B. subtilis* from environmental surface samples. Surfaces contaminated with as few as 10^3 CFU were effectively detected using this TaqMan system. We are currently designing sampling methods to increase the sensitivity of this viable methodology for the rapid and quantitative detection of spore-forming microorganisms. The use of such a system for the detection of biowarfare agents, such as *B. anthracis*, is currently being explored.